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METABOLIC MECHANISMS OF LONGEVITY: CALORIC RESTRICTION IN MAMMALS AND LONGEVITY MUTATIONS IN *CAENORHABDITIS ELEGANS*; A COMMON PATHWAY??

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ABSTRACT

Several recent studies in *Caenorhabditis elegans* have reported significant extension of the lifespan by probable loss of function mutations in various genes. When sequenced, many of these genes exhibited significant homology to genes in the mammalian insulin signaling cascade. For example, the *daf*-2 gene that has been shown to regulate lifespan in *C elegans* shares significant sequence homology with the insulin and IGF-1 receptor genes in mammals. Another longevity gene in the nematode, *age*-1, is homologous with the p110 subunit of phosphatidylinositol 3-kinase in mammals. This enzyme functions early in the mammalian insulin response cascade to influence many important cellular growth and metabolic processes. These findings and others have led to the suggestion that lifespan regulation in nematodes is controlled by a mechanism similar to that involved in lifespan extension by caloric restriction in mammals. Many intriguing similarities exist between these two model systems providing some support for this idea. However, at present there is insufficient data to conclude that similar genes or mechanisms regulate lifespan determination in nematodes and in mammals.

INTRODUCTION

Calorie restriction (CR), or undernutrition without malnutrition, remains the only nongenetic intervention that extends lifespan and retards aging and age-associated disease in mammals (1-3). Lifespan extension by CR has been reported in several species including rats, mice, hamsters, fish, spiders, *Drosophila*, and *C. elegans* (1). Laboratory rodents have been the most widely used and best characterized species in which this nutritional intervention has been studied. The lifespan extending effects of CR in rodents have been reproduced many hundreds of times in laboratories throughout the world since the landmark studies of McKay (4) in the mid-1930s.

Beginning in 1987 (5) and 1989 (6) long-term studies of CR using nonhuman primates were initiated at the National Institute on Aging (NIA) and University of Wisconsin, respectively. It remains unknown if CR extends lifespan in primates. However, the majority of published findings from these ongoing studies are in agreement with data in rodents which are well known to exhibit robust increases in lifespan on CR (reviewed in 7). Preliminary findings from the NIA suggest that deaths due to cardiovascular disease and diabetes may be reduced by CR (8). Thus it seems possible that the beneficial effects of CR may be universal among species, perhaps even humans.

Genetic approaches have been utilized in longevity studies including various genetic mutants in invertebrate models (9-13). The most reproducible of these has been lifespan extension by probable loss of function mutations in C. *elegans* including the various "*daf* and "*clk*" genes. The first of these "gerontogenes", *age*-1 (probably the same as *daf*-23), was discovered in a screen for long-lived mutants (14) and has been shown to exhibit a reduced rate of senescence resulting in extension of the wild-type lifespan (15).

The various mutations that appear to regulate lifespan in this model do so by altering metabolism, development, or both. Changes in the metabolic phenotype of the mutant worms have led several to speculate that the effects of these genetic mutations are analogous to the lifespan extension observed during CR in mammals (16-19). In addition, cloning of several of the *daf* genes has revealed significant sequence homology to genes in the mammalian insulin signal cascade further fueling the excitement about a possible relationship to CR. In fact, it has even been suggested that if some of the same genetic circuitry related to lifespan extension in nematodes also explained the CR effect in mammals, this discovery would stimulate the design of new agents capable of "tricking" mammalian cells into a dauer-like state without CR (20).

Unfortunately, the biological mechanisms that underlie the lifespan and aging retardation effects of CR remain to be fully elucidated. A leading hypothesis is that CR extends lifespan by reducing oxidative stress (21, 22). Interestingly, the majority of the lifespan extending mutations in invertebrate models have been found to be resistant to environmental stresses of various kinds, including oxidative stress. The possible link between altered signal transduction in metabolic pathways and oxidative stress resistance in the nematode is

intriguing and should be the focus of future research. However, it is not the purpose of this paper to explore this relationship, though such exploration would clearly be rewarding with respect to possible mechanisms of CR. Instead, this manuscript will explore the possible relevance of certain longevity mutations in *C. elegans* to aging, caloric restriction, and insulin signal transduction in mammals.

Longevity Mutations in *C. elegans*

Most of the lifespan mutants in nematodes are thought to regulate metabolism in some fashion. Among the best characterized of the longevity mutations are the *daf*-2, *age*-1 (*daf*-23), *daf*-16 and *daf*-18 mutants. Other mutations in *C. elegans*, such as the *clk* genes, have also been compared to CR. The present manuscript focuses on nematode mutations that have been cloned and share sequence homology with mammalian insulin signal transduction genes. The reader is referred to references 11 and 12 for more information on the *clk* mutants.

Several of the nematode mutant genes are thought to be involved in an insulin-like signaling cascade that regulates metabolism and ultimately, lifespan. These mutants, and their suggested mammalian counterparts are depicted in Figure 1. Figure 1 also illustrates one possible model for the complex interplay of the various mutants and their mammalian homologues (discussed below).

The various mutations illustrated in Figure 1 are thought to be reduction of function mutants. For example, apparent loss of function mutations in *daf*-2 or *age*-1 arrest development at the dauer stage (23). The dauer larva is an alternative third larval (L3) stage that can be induced normally by food deprivation or overcrowding and despite the fact that it represents an arrested development stage, has often been compared to CR in mammals. This comparison seems invalid since, unlike rodents on CR, dauer larvae are developmentally arrested and do not eat or grow.

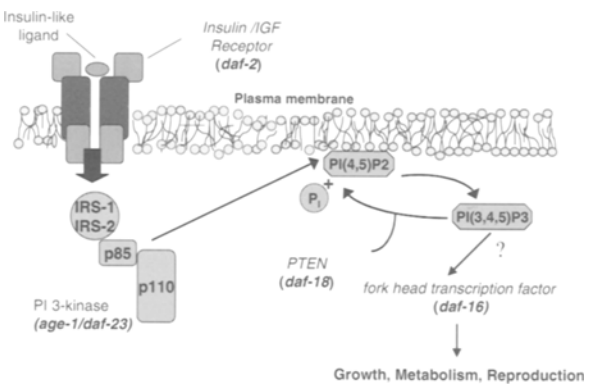

Figure 1. Figure 1 illustrates an abbreviated version of the mammalian insulin signaling cascade. Nematode genes of interest are indicated in parentheses after their mammalian homologues. The relationship of the fork head transcription factors to growth and metabolism is not yet known. The forkhead factor is illustrated to in this figure to illustrate the possible relationship based on nematode studies.

Both *age*-1 and *daf*-2 are dauer constitutive mutations that force entry into the dauer stage under otherwise normal conditions and result in increased lifespan. Weak mutations thought to involve partial reductions in activity of these genes induce dauer formation, but at a much reduced rate. These non dauer worms develop into normal adults, but with remarkably extended lifespans (24, 25). Genetic studies suggest that the *daf*-16 and *daf*-18 genes are located downstream of *daf*-2 and *age*-1 and are required for their longevity enhancing effects.

Activation of the *daf*-2 signaling cascade is thought to be important in regulating nematode growth and reproduction (26) and *daf*-2/*age*-1 mutations apparently induce a metabolic shift in the worm that has been suggested by many (9, 16-19) to be analogous to the effects of CR in mammals. The metabolic changes that occur in the mutant worms include: accumulation of fat (16), alteration of enzymes related to oxidative stress resistance and metabolism (27-30), changes in various surrogate measures of metabolic rate (25,29,31-32) and effects on fertility (18).

Figure 1 summarizes the suggested interrelationships of the various mutants. In the wild-type worm it is thought that *daf*-2 functions as a cellular receptor for insulin-like ligands. Binding to *daf*-2 then apparently activates *age*-1. Genetic studies suggest that *daf*-16 represents a negatively regulated downstream signaling output of *daf*-2/*age*-1 activation. The apparent function of *daf*-18 is to antagonize the *age*-1 signal output (26,34). Both *daf*-16 and *daf*-18 mutations suppress the lifespan extension seen in *daf*-2 and *age*-1 mutants (18,25).

Cloning experiments suggest that the four nematode genes depicted in Figure 1 are homologous with genes involved in regulation of insulin signaling in mammals. For example, *daf*-2 bears 34% and 35% homology to the insulin and Insulin-like growth factor-1 receptors in mammals (16). *age*-1 shares significant sequence homology with the 110kd (catalytic) subunit of mammalian class I phosphatidylinositol kinases (34). *Daf*-16 maps to 3 proteins in the forkhead hepatic nuclear transcription factor (HNF) family in mammals (17). Finally, molecular studies suggest that *daf*-18 is related to the mammalian tumor suppressor gene PTEN (26,33).

The fact that these lifespan regulating genes have apparently been highly conserved through evolution has led to a wide range of conclusions regarding the possible relevance of these genes to mammalian aging and age-related disease. For example, some have taken a more cautious approach and suggested, " it seems possible that elucidation of efficient life maintenance mechanisms controlled by *daf* genes could reveal insights into mechanisms affecting human lifespan" (25). Others have attempted to draw a more direct link to humans and to human aging by suggesting, "PTEN on human chromosome 10 is a candidate gene for human autosomal dominant Type II diabetes as well as for human longevity control" (26). Also, metabolic (insulin-like) control of

longevity in nematodes has been repeatedly compared to lifespan extension in mammals by CR (9, 16-19). Although intriguing, little empirical evidence exists to support the conclusion that these genes, their activities, and their ultimate downstream signaling outputs are important for determination of mammalian lifespan.

Relevance to mammals

It is reasonable to begin exploring the possible relevance of the nematode findings to mammalian aging by examining whether the signaling model proposed for the nematode system is similar to that of its mammalian homologues. Several authors have proposed the existence of a signaling cascade similar to that shown in Figure 1 (18, 26, 33). In this model binding of an insulin-like ligand to the *daf*-2 receptor activates *age*-1 and *daf*-2/*age*-1 signaling regulates *daf*-16. *daf*-16 responsive genes are thought to control metabolism, growth, and lifespan and *daf*-18 apparently limits the *age*-1 signal output.

This model is generally consistent with insulin signaling in mammals. Briefly, binding of insulin to its receptor (*daf*-2) leads to the downstream activation of phosphatidyl inositol 3-kinase (PI3-K, *age*-1). Downstream signaling outputs of this cascade (*daf* 16?) then regulate cellular growth and metabolism. The mammalian tumor suppressor, PTEN, an apparent *daf*-18 homologue, functions by limiting the signaling output of PI3-K by dephosphorylating of PI(3,4,5) P_3 (35, 36) Thus, although there remain many intermediate steps to be examined in the nematode pathway, the proposed signaling cascade bears some similarity to the insulin signal transduction pathway in mammals.

Another important exercise would be to explore whether the functions of these genes or gene products are similar in both nematode and mammalian models. Unfortunately, little is known about the proposed function of most of the mutant genes. Instead, their function has been inferred from their complex interactions and homology to mammalian genes. In mammals the converse is true in that there has been extensive research conducted on the functional aspects of mammalian insulin signal transduction. However, the possible complex genetic interactions suggested by the nematode mutation studies have not been as well studied in mammals. Nonetheless, a comparison of possible functions in worms with empirically determined functions of the mammalian genes is warranted.

It has been suggested *daf*-2 functions as a receptor for insulin-like ligands in nematodes, but it has not been reported that binding of such ligands is altered in the *daf*-2 loss of function mutants. However, evidence reported in the study by Kimura et al. (16) suggested that the predicted *daf*-2 protein possesses several structural characteristics that are similar to those reported for insulin receptors in mammals. For example, the predicted protein possesses a signal peptide, a putative ligand-binding portion, and tyrosine kinase and transmembrane domains (16). Also, mammalian insulin receptors are widely distributed in many tissues. In a similar manner, Apfeld and Kenyon (37) found that *daf*-2 was expressed in multiple tissues of *C. elegans*. Finally, several insulin-like ligands have been identified in *C. elegans*, but at least one of these does not influence dauer formation (38). Much additional work is needed to extend the genetic studies and demonstrate that the nematode genes actually perform many of the functions suggested by mutational studies. Nonetheless, to date, the findings in nematodes are consistent with *daf*-2 function being homologous with that of mammalian insulin receptors.

age-1 sequence analysis predicts a protein that is most similar to the catalytic subunit (p110) of mammalian PI3-K (34). In mammals one function of PI3-K is to phosphorylate $P(3,4)P_2$ to PIP_3 which acts via several downstream molecules to regulate a wide range of cell functions. Functional studies linking phospholipid signaling via PIP_3 to lifespan in nematodes have not been done. Studies in a variety of mammalian model systems have shown that PI3-K function and PIP_3 are involved in many cell functions (for review see 39). Several of these functions may ultimately be related to aging and lifespan including cell growth and apoptosis, stress response, glucose transport, and other aspects of cellular metabolism.

Cloning of *daf*-16 revealed its sequence homology with three members of the mammalian forkhead hepatic nuclear transcription factor (HNF) family. The highest degree of sequence homology is with the human proteins FKHR and AFX. In humans these proteins are not thought to be related to metabolic transcriptional regulation. However, HNF proteins may in part mediate insulin's effect on certain hepatic proteins (40) such as upregulation of phosphoenolpyruvate kinase in the absence of insulin (41).

Daf-18 encodes a protein homologous to the human tumor suppressor, PTEN (26, 33). As is the case for the other worm mutations, the relationship to insulin-like signaling is based entirely on genetic studies. While the normal function of the *C. elegans* PTEN equivalent is not known, its relationship to *age*-1 signal transduction is consistent with the function of PTEN in mammals. Specifically, PTEN limits PI3-K (*age*-1) signaling by dephosphorylating PIP_3, a major product of PI3-K activity.

Aging and Caloric Restriction

It is apparent that certain *daf* mutations in *C. elegans* are related to several, possibly key, components in the mammalian insulin signaling cascade. At least some of these nematode genes may subserve functions in the worm that are comparable to the functions of their mammalian homologues. Further, it is not unreasonable to suggest that altered insulin signal transduction and concomitant metabolic effects may play a key role in mammalian aging and age-related disease and perhaps the retardation of aging by CR. What is the evidence that mammalian homologues to the *daf* mutations are altered during aging and CR?

Insulin resistance increases with aging and is thought to be due to a post receptor (i.e. signaling) defect. The exact nature of this defect is not known, however, it does suggest that there may be important changes in insulin signaling that occur during aging. A review of the literature suggests that the mammalian homologues of the *C. elegans* genes may, to a limited extent, exhibit changes during aging. For example, several studies have examined receptor number and binding affinity and most report that these do not change with age in skeletal muscle (42-44). Although receptor number and binding affinity do not appear to change with age, receptor activation is reportedly reduced in older rodents (45-46). However, one study has reported no age-related change in receptor activation (47). Studies of the mammalian homologue of *age*-1 report that total PI3-K protein levels do not change in older animals (44-45). However, in response to insulin stimulation, PI3-K activity was significantly reduced in older (20 month), compared to younger (2 month) rats (45). Though limited, existing data do suggest that certain aspects of insulin signaling, specifically, receptor activation and PI3-K activity may be altered during aging.

The effects of CR on glucoregulation at the whole animal level are well documented both in rodents and in primates. Several studies have shown that CR reduces fasting glucose and insulin levels in both rats (48, 49) and rhesus monkeys (50,51) and that insulin sensitivity is increased in monkeys (50, 52). Little information is available regarding the effects of CR on insulin signaling. Receptor binding studies are inconsistent and report either no change (53-54) or increased insulin receptor binding (43) in rodent skeletal muscle. Similarly, liver binding studies are inconsistent reporting both increases (54-55) or no change with CR (48).

Only one study could be found reporting data on PI3-K changes during CR. Dean et al. (56) reported that short-term CR did not alter either total protein or insulin-stimulated activity of PI3-K. Interestingly, PI3-K activity was consistently higher in CR tissues; compared to controls at all time points (post insulin stimulation), but this effect did not reach statistical significance. Clearly, much additional work is needed to fully characterize the effect of CR on the *daf* homologues involved in mammalian insulin signal transduction and their possible role in lifespan determination. However, it remains possible that certain aspects of insulin signaling may be important in lifespan extension by CR.

In addition to molecular events implicated by genetic studies, comparison of whole animal physiological measures may offer insight into possible relevance of the *C. elegan* mutations to CR in mammals. At the outset, it is important to note that relatively few studies of this type have been conducted in the nematode model. A limited number of studies suggest that glycolysis is increased in both rodents on CR (57-59) and the nematode mutations (29). Metabolic rate and related measures have been assessed in both models as well. In rodents and monkeys there is a transient reduction in whole animal metabolic rate when CR is initiated that it not maintained after several months on the diet (discussed in 7). Reports in nematodes suggest that metabolic rate potential in *daf* mutants is increased (29). A more recent paper (60) measured metabolic rate by CO_2 production in the *daf-2* and *age*-1 mutants. As has been reported during the initial phases of CR in mammals metabolic rate was reduced in these mutatants, compared to wild type worms. Finally, studies in various species report that CR reduces total adiposity reflecting a reduction in energy storage. In marked contrast, longevity mutants in nematodes experience a metabolic shift that results in increased lipid storage (16).

It is apparent that insufficient data exist upon which to base the conclusion that lifespan extension in the nematode mutations discussed above is due to the same mechanism that produces enhanced longevity in calorically restricted mammals. The limited data are not, however, entirely inconsistent with this view. The intriguing comparisons of the two model systems are highly suggestive and support the need for additional research into this exciting area of gerontology.

Future Studies

Several important studies must be completed before conclusions regarding the relevance of the nematode studies to aging and CR in mammals can be drawn. In *C. elegans*, one of the most pressing questions to address is whether the various mutants alter food (energy) intake. Indeed, if it is found that the genetic mutations produce a CR-like reduction in energy intake in the worm, it would be exceedingly difficult to separate the effects of the loss of function mutations from those of reduce energy intake. A second focus of investigation must be to establish the functions of the various genes and gene products in the wild type worms. Specifically, it needs to be determined if the various genes, which when mutated increase lifespan, subserve the same functions in nematodes as their mammalian homologues do in mammalian model systems. For example, does *age*-1 function as an enzyme acting on membrane lipids to produce a lipid second messenger that regulates cell growth and metabolism. Once wild-type function is established then studies can focus on the role of these gene products in aging and lifespan determination.

In rodents, it is important to determine if the homologues of the various nematode mutant genes play a role in aging and lifespan extension by CR. One approach could be to develop knockout or partial knockout models for the genes in question (such as *age*-1) and determine lifespan and other hallmark effects of CR such as reduced tumor incidence. At least one study (61) has reported that a knockout model lacking the p 85 subunit of PI3-K exhibits improved insulin sensitivity, a well-known effect of CR. In addition, more extensive studies of the effects of aging and CR on insulin signal transduction are needed. In particular, it needs to be determined if CR produces changes in gene activation as predicted

by the genetic studies in nematodes. More importantly, it needs to be investigated (as the nematode studies have suggested) if CR-induced alterations in insulin signal transduction are responsible for or contribute to the lifespan extension observed in this paradigm.

In summary, various genetic mutations in the *C.elegans* model have suggested exciting possibilities regarding lifespan determination in mammals. The results of these intriguing studies have led some to suggest that a similar mechanism regulates lifespan in mutants and in mammals subjected to CR. Unfortunately, the exact biological mechanism that underlies lifespan effects of CR in mammals is not known. Further, there is insufficient data in either nematode or mammalian models to conclude that a universal mechanism operates to regulate lifespan in these diverse species. Nonetheless, the apparent conservation in mammals of several genes that regulate lifespan in the worm is exciting. Many of these nematode genes are homologous with genes in the mammalian insulin response cascade and insulin levels and insulin responsiveness are among the most consistent effects of CR. While it is tempting to suggest that longevity mutations in worms and CR in mammals regulate aging via a common pathway or mechanism, much additional work is needed before this conclusion can be drawn.

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